CLVII. THE BIOCHEMISTRY OF ALUMINIUM.

I. EXCRETION AND ABSORPTION OF ALUMINIUM IN THE PIG.

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THE biological significance of aluminium is at present an unsolved problem. Even when aluminium occurs in the diet in a readily available form, animals do not seem to utilise it in the metabolic processes to any extent; after ingestion of compounds of aluminium, the latter may be found in the organs in very small quantities, comparable to, but greater than the amounts found in the organs of similar animals receiving diets almost entirely free from aluminium. The excretion of aluminium appears to be almost exclusively by way of the alimentary canal; reports of aluminium excretion in the urine are rare.

HISTORICAL.

Smith [1900], using groups of pigs fed respectively on ordinary bread and bread made with alum baking-powder, found that the growth of the animals in each group was very similar, and after two months on this diet, failed to find aluminium in the organs. He could not detect aluminium in the urine of men or pigs fed on aluminised diets. Kahn [1911], using dogs on aluminised diet, reported that aluminium was present in the urine and also in the blood; analysis of the organs showed that the element was present in perceptible amount in the liver, spleen, muscle and kidneys. Steel [1911] also observed that aluminium was present in the blood of dogs receiving aluminium in the diet. Balls [1917] found that dogs under similar conditions of feeding absorbed aluminium, especially into the liver, but that much of this aluminium was speedily eliminated in the bile and urine. Leary and Scheib [1917] stated that puppies fed on a diet of low phosphate content to which was added a comparatively large amount of aluminium hydroxide suffered from under-nutrition, owing to the diversion of available phosphate from the urine to the faeces, with consequent lowering of metabolism. Flinn and Inouye [1928], using rats which received aluminium as an aqueous solution, reported that 70 % was excreted in the faeces and 30 % in the urine. Myers and Morrison [1928] found that dogs fed on aluminised diets showed markedly higher aluminium content in most of the internal organs than animals fed on control

diets. Underhill [1929] observed that dogs fed on diets containing aluminium showed a higher level of aluminium in the organs than dogs on control diet, but could not find any definite relation of absorption to ingestion, though the amounts found generally increased with the age of the animal. In the case of man, aluminium could be detected in the blood after a meal of aluminised food, and a slight urinary excretion (not usually exceeding 0.5 mg. in 24 hours) was sometimes observed. In a review on the occurrence of aluminium in food-stuffs by Smith [1928] possible rôles of aluminium in metabolism are considered.

PRESENT INVESTIGATION.

In these experiments the utilisation of aluminium by young pigs has been examined, and the path of excretion has been determined. An attempt has been made to ascertain if the excretion of urinary phosphorus is disturbed by the use of aluminised diets, and the amount of aluminium present in various organs has been determined.

Young pigs, recently weaned, were put on a diet known to be adequate for their growth requirements, and containing the minimum amount of aluminium; after two or three weeks' observation, to ensure that their growth and condition was satisfactory, they were divided into two groups of approximately equal weights. One group received the stock ration only and served as controls, while the other received an addition of about 1% of aluminium to the food, in the form of sodium aluminium sulphate. The groups were kept in separate pens and were not allowed out during the experiment, except for cleaning, weighing and metabolic experiments. After eight to ten weeks on their respective diets, during which period satisfactory growth took place, one animal from each group was put into a metabolic cage. When they were accustomed to the conditions, analysis of the faeces and urine was carried out to determine the daily excretion of aluminium and of urinary phosphate. When a steady excretion had been obtained for at least five days, the diets were interchanged, the control pig receiving the addition of alum, which was withheld from the experimental pig. After about ten days, the diets were again reversed and the experiment was terminated when the steady state of excretion was once more attained. The animals were fed on a progressively increasing ration until the average weight was about 80 kg. and were then slaughtered, the internal organs being reserved for analysis.

Methods of analysis. In the metabolic experiments, the faeces collected from the cage over a 24-hour period in a covered vessel were weighed and well mixed, duplicate samples of 40 g. (moist) being taken for analysis by the gravimetric method of Schmidt and Hoagland [1912] with the modification recommended by Balls [1917]. A correction was applied to the result as explained below. The volume of urine collected over the same period was determined; and a sample of 200 cc. employed for determination of aluminium [Myers and Morrison, 1928] and urinary phosphate by the Briggs method [1924].

The analysis of the organs was performed according to the method of Morrison, two duplicate samples of 40 g. (moist) being ashed at the lowest effective temperature in silica basins. In the case of the samples of stomach and intestine care was taken to free the mucosa from adherent food material by washing several times with distilled water and wiping dry with filter paper.

Correction of results obtained with the Schmidt-Hoagland gravimetric method. Preliminary tests showed that the method of Schmidt and Hoagland, determining aluminium as aluminium phosphate, could be relied on to within 1% when using pure aluminium compounds (see Table I (a) and (b)). On applying the method to the determination of aluminium in faeces it was found necessary to make a correction to the apparent amount of aluminium so determined; there appeared to be a small positive error due to the large amount of other inorganic constituents in the material, which, while insignificant in the actual sample analysed, when calculated on the whole weight of faeces over a period of several days, caused an appreciable error in the final results, which were found to be considerably too high. The errors occurring in the determination are shown in Table I (c) and (d). It was considered necessary to determine the "apparent excretion" of each pig while on control diet, and to correct suitably the figure obtained for aluminium excretion when the animal was receiving an aluminised diet. This correction was applied as shown in the tabulation of the results (Tables III and IV).

Table I. *Results of Schmidt-Hoagland gravimetric method.*

Substance analysed.	Schmidt-Hoagland method		Aluminium calculated (mg.)
	AlPO ₄ (mg.)	Al (mg.)	
Reagents and distilled water	0.7 0.3 0.3	0.008	—
(a) Potash alum. A.R. 1 g.	257.4 258.4 258.1	57.03	57.14
(b) „ 0.4 g.	102.6 103.7 102.9	22.73	22.84
(c) 40 g. faeces (pig on control diet)	3.9 4.2 4.8	0.79	0.05
(d) 40 g. faeces + 0.1 g. alum	28.9 29.2 29.1	6.42	5.71

From this table the error due to a 40 g. sample of faeces amounts to 0.75 mg. of aluminium or 19 mg. per kg. of material. The results in the metabolic experiments were of the same order, varying from 22 to 39 mg. per kg., according to daily output and percentage of moisture in the faeces. The actual aluminium content of the food, as determined by the method of Morrison, was 1.4 mg. per kg.

Experimental details.

First experiment (June–August, 1929). (a) *Feeding and growth.* This experiment was of a preliminary nature. Four pigs were used, two controls which received the standard ration and water *ad lib.*, and two experimental animals, which received in addition 25 g. of soda alum per pig per day. Growth and condition were good except in the case of one experimental pig No. 2029, which showed only slow growth. *Post mortem* examination showed that this animal had suffered from anaemia in the early stages of growth prior to the commencement of the experiment. The ration supplied had the following composition:

Barley meal	80 parts	Steamed bone flour	2 parts
Sharps	50 „	Ground limestone	2 „
Maize meal	45 „	Salt	1 part
Fish meal	20 „		

Phosphate content (P_2O_5 %) = 1.32

Aluminium content (mg. Al per kg.) = 1.4

Table II A. *Growth of pigs in first experiment (weight in lbs.).*

Diet	Control		Control	25 g. alum per day
Sex and No. of pig...	F. 2022	M. 2030	F. 2023	M. 2029
Weight				
At commencement	72	56	66	58
After 30 days	102*	89	94*	69
After 60 days	141	126	128	97
After 75 days	162	150	149	119

* Signifies that the pig was on metabolic experiment during this period.

These results indicate a slightly lower rate of weight-increase of the aluminium-fed pigs as compared with the controls, but the number of pigs under observation is too small to warrant any conclusion being drawn.

(b) *Metabolic experiment.* The results of the daily analyses of the faeces for the individual pigs are shown in Figs. 1 and 2 and in Tables III and IV (pigs 2022 and 2023). Pig 2022 received an addition of 25 g. of alum to the diet for the first time on the day before the collection of faeces and urine for the experimental period was commenced, and the addition of aluminium was continued for a total of 7 days. The analytical data for the faeces indicate that the excretion of aluminium rose to a high level within 24 hours, but rapidly diminished and returned to normal 3 days after the cessation of the aluminium feeding. Fig. 1 (on which the corrected excretion and the total ingestion are shown as rectangles) and the appropriate figures in Table III show that after making the necessary correction for "apparent excretion" on control diet, there was only a slight difference between the amount of aluminium ingested in the food and that excreted in the faeces. Pig 2023, which had been receiving an aluminised diet since the beginning of the feeding

experiment, had the aluminium removed from the diet for a period of 7 days. Fig. 2 shows that the daily faecal excretion of aluminium fell off rapidly, though traces appeared to be eliminated even at the end of the period. Hence it was not possible to obtain a factor for the "apparent excretion" on control

Fig. 1. Pig 2022.

Fig. 2. Pig 2023.

diet in the case of this pig. A factor has been calculated as an average for the other five pigs, and has been applied as a correction to the result in this case. The difference between aluminium ingested and aluminium excreted during a period of 6 days at the end of a lengthy period of aluminium feeding was only 3 %.

Table II B. *Growth of pigs in second experiment.*

Diet	Control			15 g. alum per day		
Sex and No. of pig ...	F. 2474	F. 2477	M. 2480	F. 2475	F. 2479	M. 2488
Weight						
At commencement (lb.)	54	48	65	60	49	61
After 30 days	62	60	87	82	63.5	89
After 60 days	92	69*	112	119	94	116*
After 90 days	122*	111.5	157.5	155	107	167.5
Maximum	144					
Death (108 days)	124					
Conclusion—110 days		134	183	177	135	178

* Pig on metabolic experiment during this period.

Table III. *Excretion of aluminium by pigs receiving aluminium in the diet.*

	Pig No.	2022	2477	2474
Added Al in diet per day (g.)	...	1.4340	0.7884	0.8348
Number of days on which Al was added	...	7	9	11
A. Total Al ingested (g.)	...	10.038	7.0956	9.1828
Apparent excretion of Al on aluminised diet (g.)	...	10.0171	7.1082	9.4286
Additional excretion during after-period (g.)	...	0.8234	0.2457	0.4948
B. Total apparent excretion of Al	...	10.8405	7.3539	9.9234
Apparent excretion of Al on stock diet (g. Al. per kg. of faeces)	...	0.0253	0.0398	0.0313
Wt. of faeces for experimental period (kg.)	...	14.6	6.9	14.2
C. Equivalent apparent excretion on stock diet	...	0.3694	0.2746	0.4445
B-C. Corrected excretion	...	10.4711	7.0793	0.4789
Difference between corrected excretion and total ingestion A	...	0.4331	-0.0153	0.2961
Difference %	...	4.3	-0.1	3.2

Table IV. *Excretion of aluminium in faeces of experimental pigs receiving aluminium.*

	Pig No.	2023	2488	2475
Al ingested per day (g.)	...	1.434	0.7884	0.8348
Number of days for which total Al excretion was determined	...	6	7	7
A. Total Al ingested (g.)	...	8.604	5.5188	5.8436
B. Total excretion for experimental period	...	9.1879	5.6253	5.8771
Apparent excretion of Al in faeces on control diet (g. per kg.)	...	0.0305	0.0337	0.0225
Total weight of faeces for experimental period (kg.)	...	10.2	8.1	10.4
C. Equivalent excretion of Al on control diet (g.)	...	0.3111	0.2730	0.234
B-C. Corrected excretion for experimental period (g.)	...	8.8768	5.3523	5.6431
Difference between corrected excretion and total ingestion	...	0.2728	-0.1665	-0.2205
Difference %	...	3.0	-3.0	-3.8

Second experiment (December, 1929–April, 1930). (a) Feeding and growth.

The results obtained in Exp. 1 seemed of sufficient interest to warrant a further trial. Accordingly six pigs were selected and divided into two groups as before. The same basal ration was used, but in this case the experimental animals received 15 g. of alum per day. The conditions and time of year were adverse to good growth, and after the sixth week of the experiment an addition of separated milk at the rate of 1 lb. per lb. of ration was made, which improved progress. One of the control pigs died of pleuro-pneumonia a few days before the experiment was concluded; there had been no previous record of illness, and all the other animals remained healthy throughout.

The weight records show that the aluminium-fed pigs in this case did as well as, if not slightly better than, the controls. Taking the two experiments together it would appear that aluminium fed at the levels adopted had no particular effect on the growth or health of the animals. It was noticeable that the pigs on the aluminium diet gave a considerably larger weight of faeces per pound of food ingested, on the average about 50 % more as determined during the metabolic experiments. But the transference of the aluminium to the control pigs for periods of 7 days or more did not bring about any change in the moisture content of the faeces of either the control or experimental animal.

(b) *Metabolic experiments.* The results of four metabolic experiments are set out in Tables III and IV (pigs 2477, 2474, 2488, 2475). Pigs 2474 and 2475 received in place of alum during this period aluminium phosphate in the form of a decomposed baking powder (Calumet brand) at the rate of 40 g. per day, which yielded approximately the same quantity of aluminium. In all cases there is close agreement between the aluminium ingested and that excreted in the faeces during the experimental period. The actual difference is of the order of 0.02 g. of aluminium on an ingestion of 0.8 g.—a difference within the limits of probable error for sampling and analysis. In the control animals, the rapid rise in aluminium excretion within 24 hours of ingestion and the rapid diminution and return to normal level within 3 days of cessation, are again notable.

Urinary excretion of aluminium.

In the above balance figures no account has been taken of the urinary excretion of aluminium. The actual figures for such excretion in the case of the six pigs used in metabolic experiments are given in Table V.

Table V. *Aluminium found in urine during metabolic experiments.*

	Pig No.	2022	2023	2477	2488	2474	2475
A. <i>Control diet:</i>							
Days on diet		14	7	12	10	12	10
Days on which Al was found		3	2	5	2	7	4
Total Al found (mg.)		15.7	6.1	4.2	0.9	4.3	3.6
B. <i>Aluminium diet:</i>							
Days on diet		7	14	9	10	11	13
Days on which Al was found		1	4	3	4	6	8
Total Al found (mg.)		3.0	51.0	1.6	2.6	6.3	6.2
Aluminium found over whole period (mg.)		18.7	57.1	5.8	3.5	10.6	9.8

It seems probable that this aluminium found in the urine, though small in amount, was derived from faecal contamination, a process difficult to prevent entirely. The following considerations favour this conclusion. The urine was, in the majority of cases, quite clear and apparently free from suspended matter, and in these cases it was not filtered; sometimes it contained aluminium, but more usually it did not. In other cases, where the urine obviously contained suspended matter, it was filtered to remove this. The solids were finely divided, as quick filter papers did not hold them back, and it was necessary to use No. 50 Whatman (hardened) papers and suction to obtain

a clear solution. After such filtration, the urine never contained aluminium. The amount of material left on the filter was negligible and could not have added noticeably to the weight of aluminium in the faeces, if transferred to it.

Urinary excretion of phosphorus.

The results for excretion of urinary phosphorus are given in Table VI, and show no clear difference between the phosphate output of the control and experimental animals before or after the change in diet. There is no evidence of any diversion of phosphorus from the urine as the result of addition of aluminium in the form of alum.

Table VI. *Urinary output of phosphate of pigs on control and aluminium diets.*

Pig No.	Wt. of pig kg.	Aluminium compound added to diet	Phosphate output in urine	
			Control diet	Aluminium diet
2477 (control)	30	Soda alum	1.07	1.09
2488 (experimental)	42	"	1.41	1.22
2474 (control)	39	Aluminium phosphate	1.71	1.8
2475 (experimental)	53	"	2.32	2.20

Aluminium content of animal organs.

When the animals in the various experiments were slaughtered, the liver, lungs, heart, small intestine and stomach were analysed to determine the aluminium content, with the results shown in Table VII.

Table VII. *Aluminium content of the organs of pigs on control and aluminised diets.*

	No. of pig	Al fed per day (g.)	Days fed	Aluminium content (mg. per 100 g. of fresh material)				
				Liver	Lung	Heart	Small intestine	Stomach
A. Control pigs								
2030	0	—	—	0.47	0.17	0.55	0.75	0.62
2480	0	—	—	0.83	0.15	0.75	0.72	0.95
2022	1.4	7	7	0.75	0.14	0.50	0.80	0.12
2474	0.83	10	10	0.59	—	—	0.25	—
2477	0.78	9	9	0.84	0.47	0.45	0.24	0.28
B. Experimental pigs								
2023	1.4	70	70	0.97	0.24	0.72	0.62	0.80
2029	1.4	70	70	1.9	0.16	1.25	0.93	0.88
2475	0.8	100	100	0.98	0.31	0.44	1.30*	0.23
2479	0.8	100	100	0.86	0.24	0.55	1.50*	0.30*
2488	0.8	100	100	1.04*	0.17	0.94	0.92	0.81
Average—5 control pigs				0.69	0.23	0.56	0.55	0.49
Average—5 experimental pigs				1.15	0.23	0.78 (0.66)	1.05	0.60

* Indicates result of one sample. The remainder were in duplicate.

These figures show that there is a definite increase in the aluminium content of the liver and small intestine as a result of aluminium ingestion but that there is no marked rise in the other organs, the high figure for the heart in the experimental group being due to an aberrant figure from pig 2029. The results for stomach material are irregular.

DISCUSSION.

The daily excretion of aluminium indicates very clearly that faecal excretion rapidly falls to a negligible amount when aluminium is withheld. The delayed excretion is small in amount and seems to be due to mechanical obstruction in the intestine; it can hardly be accepted as evidence of the elimination of absorbed aluminium. But the method does not take account of small quantities, which might be absorbed and excreted without appreciably affecting the results. A more effective argument against absorption is the relatively small excretion of aluminium in the urine, or absence of excretion when the urine has been filtered to remove traces of faeces. The urinary excretion of phosphate disposes of the very doubtful assumption that aluminium may affect the metabolic processes by combining with available phosphate in the intestinal tract and thereby immobilising it. A further contention against absorption of aluminium to any great extent is afforded by the very small amount of the element found in the organs most likely to be affected by any absorption when on an aluminised diet for a period of 70 days and upwards.

SUMMARY.

- (a) Intake and excretion of aluminium in the pig are equal (within the limits of experimental error).
- (b) Aluminium excretion is confined solely to the alimentary tract.
- (c) The absorption of aluminium from normal diets containing moderate amounts of available aluminium, as determined by the analysis of the organs, is small.
- (d) Diversion of phosphates from the urine to the faeces does not occur on such diets.
- (e) No harmful effect on general growth and metabolism results from feeding comparatively large amounts of aluminium.

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